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     (FILE 'HOME' ENTERED AT 11:12:08 ON 01 APR 2002)

     FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 11:12:24 ON 01 APR 2002
L1           342 S SYBR (W) GREEN AND (PCR OR POLYMERASE(W)CHAIN OR MELTING (W)
L2            60 S L1 AND PY<1998
L3            30 DUP REM L2 (30 DUPLICATES REMOVED)

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DOCUMENT-IDENTIFIER: US 6323337 B1
TITLE: Quenching oligonucleotides

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DEPR:
Ohgonucleotide primers labeled with quencher dyes, prepared as described above (Example 8), are separated by electrophoresis in polyacrylamide or agarose gels, or are separated in capillary electrophoresis, or using microfluidic methods, along with unlabeled primers and PCR products or other products of primer extension. The gels are then stained with an appropriate nucleic acid gel stain, such as ethidium bromide, SYBR Green I stain, SYBR Green II stain, a red fluorescent SYTO stain, SYBR Gold stain, SYTO blue stain, SYTO green stain, or SYTO orange stain. Alternatively, the capillary electrophoresis is performed min the presence of the stain, using standard methods. Primers labeled with the quencher dyes are essentially nonfluorescent in the presence of the fluorescent nucleic acid stain, and thus do not contribute appreciably to the staining pattern in the gel. This simplified pattern facilitates automated gel or capillary electrophoresis analysis. Similarly quenched primers or ligation monomers can be eliminated from the staining pattern in a ligation assay containing one labeled and one unlabeled oligonucleotide, or a telomerase assay. Primer dimers with quenchers on both 5' ends are also not detected by fluorescence because their fluorescence is essentially fully quenched, so that even if they are abundant they do not obscure signals due to short amplification products.

DEPR:

PCR reactions are prepared, using oligonucleotide primers labeled with quencher
Compound 16 or 19. SYBR Green I stain is included at a dilution of 1:50,000 of
the commercially available stock solution, or PICOGREEN reagent is added to the
reaction after PCR is completed at a final concentration of 0.8 .mu.M, and the
fluorescence of the solution is measured. If SYBR Green I stain is included in
the reaction, then the reaction can be monitored in real time, using an
appropriate instrument, such as the LIGHTCYCLER (Roche) or the GENEAMP 9700
(Perkin Elmer). The background fluorescence in reactions containing quenched
primers is lower than that observed in those containing unlabeled primers, and
in addition, primer dimers do not contribute to the product signal. Other
stains, such as YOYO-1 or OLIGREEN reagent, are added to the solution after PCR
with the same results. Other stains, such as YO-PRO-1, are added to the
solution prior to or during PCR with essentially the same results.

DETL:
TABLE 3 Fluorescence quenching by selected quenched oligonucleotides when
associated with selected nucleic acid stains. Relative Fluorescence.sup.1
Quenching Moiety Nucleic acid Ex/Em.sup.2 Free stain (nm) dye.sup.3 DABCYL
Cpd. 6 Cpd. 8 Cpd. 11 Cpd. 14 Cpd. 16 Cpd. 19 POPO-1
434/456 2.6 8.7 10 6.1
3.5 6.1 PO-PRO-1 435/455 9.9 23 21 25 21 22 SYTO 43
438/460 35 39 35 43 41 44
BOBO-1 462/481 16 30 40 33 41 27 SYBR Green I 494/521 3.7
7.9 4.9 5.1 4.0 6.0
2.7 28 PicoGreen reagent 493/525 3.2 9.4 5.3 4.2 3.6 6.8
4.0 29 OliGreen
reagent 498/515 4.3 9.6 7.1 5.2 4.3 6.4 4.2 27 SYBR Gold
stain 494/530 3.2
7.7 4.0 4.0 4.0 5.3 YO-PRO-1 491/509 4.3 16 9.9 8.8 6.5
8.9 6.0 34 YOYO-1
491/509 13 24 23 11 12 9.2 8.9 9.2 Ethidium bromide
518/605 95 99 87 97 86 89

75 83 JOJO-1 529/545 1.7 2.8 1.4 BOBO-3 570/604 76 60 56 64 60 64 YOYO-3
612/631 6.9 38 28 8.9 5.2 5.1 SYTO 59 630/645 9.1 61 27 15 6.3 11 SYTO 61
630/645 12 60 37 23 6.9 12 .sup.1 Relative fluorescence is the percentage of
fluorescence exhibited by an quenching moiety-labeled oligonucleotide stained
with the indicated nucleic acid stain relative to that of an unlabeled
oligonucleotide of the same sequence, stained with the same amount of nucleic
acid stain. .sup.2 Ex/em designates the fluorescence excitation and emission
maxima for the indicated nucleic acid stain, bound to double-stranded DNA.
.sup.3 Free dye indicates the relative fluorescence exhibited by the nucleic
acid stain alone, expressed as a percentage of the fluorescence of the
unlabeled oligonucleotide of the same sequence, bound to the same amount of
nucleic acid stain. In some cases, the binding of particular quencher
conjugates reduced the fluorescence of the nucleic acid stain solution to a
level lower than that observed for free, unbound stain.

DOCUMENT-IDENTIFIER: US 6316198 B1
TITLE: Detection of mutations in genes by specific LNA primers

---------- KWIC ---------

DRPR:
FIG. 6. Melting curve analysis of PCR fragments generated with the
EQ3053/EQ3213 primer set and either the "A-allele" template (A, lower curve) or
the "G-allele" template (G, upper curve). The upper panel shows the
fluorescence of the SyBR Green I dye bound to double-stranded amplicon. The
lower panel shows the first negative derivative (-dF/dT) of the melting curve
in upper panel. The T.sub.m is seen as the peak on the -dF/dT plotting.

L Number	Hits	Search Text	DB	Time stamp
1	130	sybr near3 green	USPAT; US-PGPUB	2002/04/01 11:04
2	36	(sybr near3 green) same (allel$ or polymorphism or oligonucleotide)	USPAT; US-PGPUB	2002/04/01 11:05